Development of a Meridic Diet for *Hylobius transversovittatus* (Coleoptera: Curculionidae) and the Role of Carbohydrates in Feeding, Growth, and Survival of Larvae

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ABSTRACT The root-feeding weevil *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae) is used for biological control of the invasive plant purple loosestrife, *Luthrum salicaria* L. (Lythraceae). A simple rearing system for this weevil was developed with the goals of improving production techniques and increasing the availability of insects for field introduction. Additionally, the dietary effects of digestible and indigestible carbohydrates were explored. A meridic diet for rearing *H. transversovittatus* was formulated through nutritional alterations of a boll weevil, *Anthonomus grandis grandis* Boheman, diet. Diet attractiveness was evaluated on two levels: first, by recording the incidence of initial tunneling, and second, by estimating the larval establishment rate. The performance of test diet formulations was further assessed by measuring developmental and survival rates of *H. transversovittatus*. Sucrose, starch, and three types of indigestible carbohydrates were tested as components to improve diet performance. Physical properties of the diet, modified by fillers in test formulations, produced major effects on the initial tunneling of hatchlings. The establishment of hatchlings was affected by chemical properties of the diet. Increases in sucrose concentration decreased larval establishment, decreased the rate of larval development, and decreased larval survival. However, omitting sucrose from the diet, or replacing it with starch, increased mortality of first instars. In advanced stages of larval development, omitting sucrose from the diet did not significantly affect larval survival. The developmental rate of larvae was increased when the amount of digestible carbohydrate was reduced. To date, seven generations of the univoltine *H. transversovittatus* have been successfully produced on this new meridic diet.

KEY WORDS root, weevil, rearing, diet, nutrition

The root-feeding weevil *Hylobius transversovittatus* Goeze (Coleoptera: Curculionidae) was introduced into North America in 1992 as a biological control agent of purple loosestrife, *Luthrum salicaria* L. (Lythraceae) (Hight et al. 1995). *H. transversovittatus* was chosen as a biological control agent because larvae are very destructive to the root system where they deplete below ground resources, disrupt the vascular system of the plant, and open wounds to saprophytic organisms that can further damage the plant (Blossey 1993, Blossey et al. 2000). Adults feed above ground on leaves and shoots, and females oviposit into stems or roots. Early larval stages feed in the stem before burrowing into roots where they feed and pupate. Eclosed *H. transversovittatus* adults tunnel to the soil surface. Depending on the time of oviposition, *H. transversovittatus* may produce one or two generations per year (Blossey 1993).

Adults are nocturnal, hiding during the day under plant debris. This obscure life style makes collecting these agents for biological control redistribution difficult and expensive. To help overcome this problem, Blossey et al. 2000 developed a rearing system for *H. transversovittatus* based on a semiartificial diet. This diet was largely composed of ground purple loosestrife roots, which were specially prepared for feeding *H. transversovittatus* larvae. Although successful, this rearing system required time and labor for harvesting and handling plant material as well as expensive equipment for root preparation, which is labor-intensive and unpractical for small insectaries.

For insect rearing, the use of meridic diets has many advantages over diets containing host plant materials because production is easier, quality is uniform, and rearing can take place throughout the year. However, artificial diets should be based on the nutritional requirements and feeding behavior of the insect. Purple loosestrife is a perennial wetland plant. It was introduced to North America from Europe, where it established along the northeastern coast early in the nineteenth century (Thompson et al. 1987). These herbaceous plants spread to become a major weed throughout the northeastern, midwestern, and western United States. Purple loosestrife forms a woody

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crown from which new shoots arise every year (Shamsi and Whitehead 1974). Like many other temperate perennials, it accumulates storage carbohydrates in the roots in late summer and fall as an energy source for spring shoot development. Roots of purple loosestrife contain soluble sugars (sucrose, fructose, and glucose), starch, and structural carbohydrates (Katovich et al. 1998).

The main objective of this work was to develop an economical artificial diet for *H. transversovittatus* larva by eliminating the need for host plant material. A secondary objective was to learn more about the nutritional and phagostimulatory requirements of immature *H. transversovittatus* to facilitate diet development for *H. transversovittatus* that may apply to other root-feeding insects.

In this study, the role of carbohydrates was investigated because they are present in large quantities in the insect's natural diet (Katovich et al. 1998), and they are known to affect chemical and physical attributes of artificial diet (Dadd 1985). Sucrose was tested in this study because of its phagostimulatory characteristics and nutritional properties (Chippendale and Reddy 1974). These studies were followed with experiments involving bulking agents because they affect physical properties of the diet. It has been recognized that bulking agents can impact gut residence time (Waldbauer 1968, Cohen 2004). The weevil's natural substrate (root) is rich in undigestible carbohydrates (Katovich et al. 1998); therefore, the inhibition of nutrient absorption by the addition of bulking agents seemed appropriate. Starch was included in this study because it is found in high concentrations in purple loosestrife roots (Katovich et al. 1998).

Studies presented here focused only on carbohydrates; however, studies on other nutrients also were necessary to improve diet performance, and they are still in progress. A plant material-based diet was not included as a comparison in this study because the presented diets do not fully represent the final potential effectiveness of a meridic diet. Such a comparison will be made upon final diet formulation. This work, however, identified key components that allowed first a successful meridic diet to be developed for *H. transversovittatus* larvae.

Materials and Methods

Assessments of test diets included a number of different measures of effectiveness to evaluate impact on specific biological processes. These measures included hatching rate (percentage of eggs placed on the diet surface that hatched), tunneling rate (percentage of hatchlings that tunneled into the diet), establishment rate (percentage of larvae that tunneled and stayed in the diet), survival rate, and physical measures of the growth rate (width of head capsule) of *H. transversovittatus.* Evaluation of the incidence of initial tunneling was based on the presence or absence of tunnel openings, independently of the location or condition of the larva at the time of inspection. Establishment of the larvae was recorded 6 d after hatching or later, and it was based on the number of individuals that stayed in the diet and continued to feed. Head capsule width was used as a measure for assessing the developmental stage. Long-term survival was assessed in most experiments 130 d after hatching because most of the insects reared on the control diet in our experiments tended to complete development within 110 d with a mean developmental time of 90–100 d.

Table 1. Gast boll weevil diet, used as the starting diet for modification to rear *H. transversovittatus*

Dry ingredient	(g)	Liquid	(ml)
Pharmamedia	63.46	Water	860.00
Isolated soy protein	22.66	Corn oil	1.89
Corncob grit	11.33	HCl	0.8
Sucrose	15.86		
Wesson's salt	2.49		
Cholesterol	0.68		
Ascorbic acid	1.71		
Potassium sorbate	1.13		
Methyl paraben	0.79		
Vitamin mix	2.49		
Agar	18.01		

Read table entries from left to right within a row.

Insects. A laboratory colony of *H. transversovittatus* was started with field-collected adults imported from Germany. Adult HT, with 75–100 individuals per cage (50 by 38 by 45 cm), were kept in growth chambers at constant conditions of 25 ± 1°C and a photoperiod of 16:8 (L:D) h. Adults were fed shoots of purple loosestrife grown under standard greenhouse conditions. Shoots were placed into "feeding and ovipositing stations" (500-ml round plastic food containers with indented lids). Into each lid (11 cm in diameter), three to four holes (1 cm in diameter) were cut. Four 1–2-mm-thick pieces of florist foam were placed into the lid indentation for oviposition. Loosestrife shoots were pushed through foam, and holes on the lid into the container filled with water. Females laid eggs between the foam slices close to the plant stems. Eggs were collected twice weekly, and they were kept at 10°C for up to 4 d until experimental use. Eggs were surface sterilized with 0.002% benzalkonium chloride (ICN, Aurora OH), rinsed in sterile distilled water, and placed on the surface of test diets for hatching.

Diet. The initial medium for our experiments was selected based on the hypothesis that many insects, including root-feeding weevils, have similar nutrient requirements (Fraenkel 1959). Thus, any diet that is palatable to the first instars of *H. transversovittatus* also may be an acceptable medium for further modifications to meet the nutritional requirements of older larvae. Additional selection factors were the cost-effectiveness of labor, equipment, and ingredients. The Gast boll weevil, *Anthonomus grandis grandis* Boheman, diet (BWD) (Roberson and Wright 1984) (Table 1) was selected as our starting point based on pilot tests with *H. transversovittatus* larvae. The initial BWD was not adequate for rearing and survival, but it was not rejected by first instars, because some feeding and development occurred.

Most of the ingredients for the diet were industrial grade products obtained from Bio-Serv (Frenchtown, NJ) or directly from the producers. They included Grit-O'Cobs mesh size −60 (fine) and 40/60 (coarse) (The Andersons, Inc., Delphi, IN); Toasted Nutrisoy Flour (ADM, Decatur, IL); Pharmamedia (Traders Protein, Memphis, TN); Mira-Gel 463 starch (A.E. Staley, Decatur, IL); TerraVet oxytetracycline HCl (Pfizer, New York, NY); and isolated soy protein (John R. White Company, Inc., Birmingham, AL). Chemical ingredients were ordered from the Sigma-Aldrich (St. Louis, MO) (Table 1).

Equipment. All mixing bowls, spatulas, and supporting equipment were sterilized with a 10% Clorox solution immediately before use. The diet was mixed in 2-liter batches by using a Waring commercial blender. The prepared diet was poured under sterile conditions within a clean-air bench into rearing trays (BIO-RT-32, CD International, Pittman, NJ) by using a rate of 18 ± 2 ml per tub with a 500-ml plastic squirt bottle as an applicator. Trays (44 by 21 cm) contained 32 individual "tubs" (32 cm^3 each) with adhesive covers.

The test diets were prepared in the same manner for all experiments. A precision balance was used in measuring solid ingredients. An Eppendorf automatic pipette was used to measure and transfer liquid ingredients. With the exception of the vitamins, antibiotics, and agar, the dry ingredients were mixed thoroughly before placement in the diet mixer. The mixed dry ingredients were mixed in the blender with one half of the required volume of 65°C tap water. The remaining water was combined with agar and heated to boiling in a microwave oven. The agar was allowed to boil two times before adding it to the mixture in the blender. The ingredients were blended for 30 s. The vitamins and antibiotic were then added and blended for another 2 min until completely incorporated into the diet mixture. The diet was than transferred to the applicator and immediately dispensed into trays. The uncovered trays were left on the clean bench for three hours to cool before inoculating with eggs.

Experiments. One ingredient at a time was varied in the experimental diets for comparison with control diets. Each improvement in the diet was made stepwise from the initial BWD (Table 1) based on results of previous experiments. Six stepwise experiments are reported here. Sample sizes and number of replicates per experiment were adjusted as needed in each test to ensure statistical validity.

H. transversovittatus eggs were placed on both experimental and control diets on the same day for each experiment. One *H. transversovittatus* egg was placed on the surface of the diet, and if more than one egg was inoculated, it was included in the description of that specific experiment. In experiments where initial tunneling was not being assessed, the diet surface was scratched or pierced with a sterile pipette to facilitate burrowing of neonate larvae.

Nutritional and Behavioral Effects of Sucrose Concentrations (Experiment 1). Four sucrose levels in the diet were compared for diet attractiveness, weevil establishment, rate of larval development, and survival. For each treatment, three replicate trays consisting of 32 individual diet tubs were filled with the experimental diet containing 0.9, 3.6, or 7.2% sucrose, and they were compared with BWD containing 1.8% sucrose. Samples consisting of 16 randomly selected individuals per treatment were taken at 3, 6, 12, 24, 48, and 96 d after hatching and assessed. Larvae (alive and dead) were located and removed from diet, and head the capsule width was measured. During sample day 3 and 6, a bioassay for diet attractiveness was assessed using a nonchoice measure of feeding response and by a visual assessment of the insect's location in the diet.

Effect of the Addition of the Bulking Agent (Experiment 2). Four cellulose levels (0, 16, 24, and 36%) in the diet were compared using 16 randomly selected individuals per treatment as samples. Establishment was assessed 11 d after inoculation. Larval survival and the effect on the larval developmental rate were evaluated 72 d after inoculation. Increased contamination occurred in this experiment, leading to the introduction of antibiotics to the diet. TerraVet oxytetracycline HCl was added in 0.1% concentration to all treatments in the following experiments. The selection of this antibiotic was made based on experience from boll weevil rearing, where this type of antibiotic formulation was successfully used when contamination was a problem.

Effect of Replacing Cellulose with Corncob Grit (Experiment 3). This experiment was designed to replace cellulose (additional bulking agent added in experiment 2) with corncob grit (already present in BWD) with the goal of simplifying diet preparation. Four diets with different filler combinations were compared for egg hatching, tunneling, and establishment rate 11 d after inoculation. The standard BWD was maintained as the control diet. Tested bulking agents were cellulose and two different mesh sizes of Grit-O'Cobs (fine and coarse corncob grit). The bulking agent (118.5 g) was added to 1,000 g of the control diet for each treatment and four replicates of 16 tubs were inoculated with sterilized eggs. Assessments of the test diets were done by previously described procedure. Because we were able to rear *H. transversovittatus* from egg to adults only in the diet containing cellulose as the bulking agent, we accepted the BWD with an addition of 118.5 g of cellulose per 1,000 g as the next control diet (now identified as the cellulose weevil diet CWD).

Effect of Corncob Grit Exclusion (Experiment 4). The bioassay to assess the effects of omitting corncob grit from the control diet was designed using a treatment diet (CWD-CG) that did not contain corncob grit. This diet was compared with the CWD (control) containing fine corncob grit. One tray containing 32 tubs per treatment was inoculated with weevil eggs, and the entire setup replicated three times. Previously described measures of effectiveness were applied.

Effect of Incorporating Soy Flour versus Pharmamedia (Experiment 5). An experiment was conducted to test the use of a soy based product Toasted Nutrisoy Flour in place of cotton-based Pharmamedia. The goal was to increase first instar survival. Trays containing

Table 2. Dry ingredients for diets used in experiments 5 (CWD and SFWD) and 6 (SFWD, cellulose SFWD, and starch SFWD)

CWD	(g)	SFWD	(g)	Cellulose SFWD	(g)	Starch SFWD	(g)
Sucrose	15.86	Sucrose	15.86	Cellulose	15.86	Starch	15.86
Pharmamedia	63.46	Soy flour	63.46	Soy flour	63.46	Soy flour	63.46
Isolated soy protein	22.66		22.66		22.66		22.66
Corncob grit	11.33		11.33		11.33		11.33
Wesson's salt	2.49		2.49		2.49		2.49
Cholesterol	0.68		0.68		0.68		0.68
Ascorbic acid	1.71		1.71		1.71		1.71
Potassium sorbate	1.13		1.13		1.13		1.13
Methyl paraben	1.58		1.58		1.58		1.58
Vitamin mix	2.49		2.49		2.49		2.49
Cellulose	118.50		118.50		118.50		118.50
Agar	18.01		18.01		18.01		18.01

Read table entries from left to right within a row. CWD is final control diet that best supports complete development of *H. transversovittatus* larvae. Liquid ingredients (not presented) are the same as in Table 1.

CW-diet (Table 2) and trays containing the experimental soy flour diet (SFWD) (Table 2) were inoculated with weevil eggs by using the same procedures as in previous bioassays. The experiment was replicated five times. In addition to the standard measures, the mortality of first instars was assessed independently of their tunneling behavior and establishment. Based on this experiment, toasted soy flour (SF) was accepted as the standard ingredient for the diet formulation SFWD, and it used as the base diet for experiment 6.

Effects of Different Carbohydrates (Experiment 6). An experiment comparing the impact of three different carbohydrate sources on the initial tunneling of first instars, larval mortality, adult size and developmental rate was designed using 1) diet prepared with a digestible oligosaccharide, sucrose (SFWD); 2) diet prepared with a digestible polysaccharide (starch SFWD); and 3) undigestible carbohydrate diet (cellulose SFWD) (Table 2). Each carbohydrate diet contained 15.86 g of test compound per 1118.5 g of diet. For all prepared diets, vitamins (normally combined with sucrose) were mixed with cellulose to eliminate sucrose from the vitamin mix. One tray per treatment (32 tubs each) was inoculated with two surface-sterilized eggs per tub, replicated four times (four trays of 32 tubs each) and incubated until assessment. Larval establishment was assessed 14 d after inoculation. At 130 d, the contents of all tubs were carefully dissected and tunneling activity, larval mortality, developmental rate, survival, and adult size were assessed. Data were analyzed based on the number of insects that were recovered from the diet.

Statistics. Statistical analysis was performed using the SPSS 10.0 statistical package (SPSS Inc., Chicago, IL). General linear regressions were performed on all concentration related assays to assess correlations, trends, and significant relationships. For all binomial tests (two-diet comparisons), the proportion as it differed from the control was used as the expected value for each experimental treatment. Arcsine transformations were performed where appropriate on data involving percentages or proportions before the use of analysis of variance (ANOVA) or a general linear model (Sokal and Rohlf 1969).

Results

Experiment 1. This test showed that the percentage of larvae that burrowed into the diet was similar on all tested sucrose concentrations ($F = 0.0247$; df = 3, 60; $P = 0.836$; Fig. 1A). The general trends of establishment changed during the period 3–6 d after hatching (Fig. 1B). Samples collected 6 d after hatching showed a significant negative correlation between the sucrose concentration in the diet and establishment ($F = 4.674$; df = 3, 60; $P = 0.005$; Fig. 1B).

Overall larval mortality assessed over a period of 96 d did not significantly differ between the treatments ($F = 2.344$; df = 3, 380; $P = 0.114$). Mortality of larvae increased with time (Fig. 2), and it was significant ($F = 117.50$; df = 19, 300; $P < 0.0005$). No pupation was observed in this experiment, but head capsule measurements revealed that the rate of development was negatively affected by the increase of the dietary sucrose concentration ($F = 13.526$; df = 3, 17; $P < 0.0005$; Fig. 3). The samples collected 46 and 96 d after hatching were small due to high mortality (87–100%) (Fig. 2). Tukey's multiple comparison test showed that the width of the head capsule was similar in larvae reared on the diet with 0.9 and 1.8% sucrose ($P = 0.960$) but smaller in individuals reared on diets containing 3.6 and 7.2% sucrose ($P < 0.0005$). These results lead to experiment 2 and the hypothesis that reducing carbohydrate concentration, and possibly all nutrient levels, would improve diet performance.

Experiment 2. An analysis of establishment showed significant differences between the control diet (0% cellulose) and treatments (16, 24, and 36% cellulose) (Fig. 4A). Addition of different concentrations of cellulose indicated larval establishment was significantly improved by increasing the amount of cellulose ($P < 0.0005$; binomial one-tailed test). Survival and the rate of development were assessed 72 d after egg inoculation. Samples from the 24% cellulose concentration treatment were discarded due to diet contamination; thus, results contain information from the BWD control (0% cellulose) and the remaining two (16 and 32%) cellulose treatments. No difference was found in the survival rates of larvae reared on different diets. However, head capsule width, as a measurement of

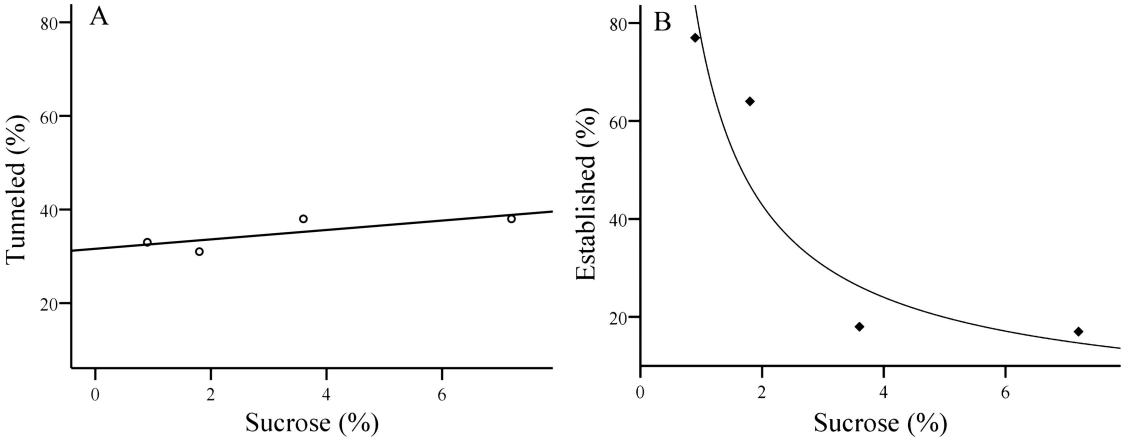

Fig. 1. Effect of sucrose concentration in BWD on initial tunneling ($y = 0.951x + 31.446$; $r^2 = 0.667$) (A) and establishment ($y = 84.155x^{-0.844}$; $r^2 = 0.869$) (B) of newly hatched larvae of *H. transversovittatus*.

the developmental rate, showed significant differences ($F = 9.431$; df = 2, 24; $P = 0.001$). Dunnett's (two-sided) comparison test shows that the head capsules of larvae reared on the control diet (0% cellulose) were smaller ($P = 0.005$ for 16% and $P = 0.001$ for 32% cellulose treatments) (Fig. 4B) than those reared with the cellulose addition.

Experiment 3. A comparison of cellulose verses corncob grit as bulking agents indicated that the egg hatching rate was not affected by either filler compared with the original BWD ($F = 0.935$; df = 3, 233; $P = 0.424$; Fig. 5). Tunneling behavior was affected by adding fillers ($F = 9.936$; df = 3, 233; $P < 0.0005$). Tukey's honestly significant difference (HSD) multiple comparison test shows that the addition of cellulose and coarse corncob grit increased first instar tunneling (for cellulose, $P = 0.001$ and for coarse corncob grit, $P < 0.0005$), but this was not the case in the fine corncob grit treatment ($P = 0.891$; Fig. 5). Consequently, a significant difference in tunneling activity was found between the fine and coarse corncob grit treatments ($P = 0.001$).

Differences in the establishment of first instars occurred between treatments of filler type, with higher establishment in the cellulose treatment ($F = 5.123$; df = 3, 123; $P = 0.002$; Fig. 5). Furthermore, multiple comparison tests, run against BWD, revealed a significant difference only for the cellulose treatment ($P = 0.001$), whereas no differences were found for either of the corncob grit treatments ($P = 0.154$ and $P = 0.235$). Moreover, when corncob grit treatments (fine and coarse) were compared, no difference was detected ($P = 0.951$; Fig. 5).

No destructive sampling was performed in this experiment; thus, all inoculated samples were retained to evaluate diet performance through adult emergence. The first adult male eclosed in 61 d, and the first and only female took 93 d to complete development.

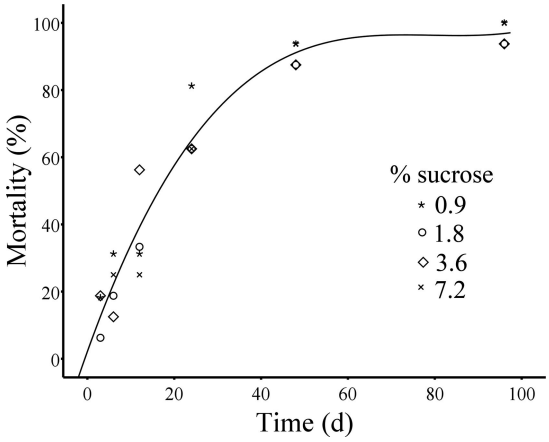

Fig. 2. Mortality of larvae reared on diets containing different sucrose concentrations assessed 3, 6, 12, 24, 48, and 96 d after hatching ($y = 2.16 + 3.608x - 0.46x^2 + 0.0000192x^3$; $r^2 = 0.949$).

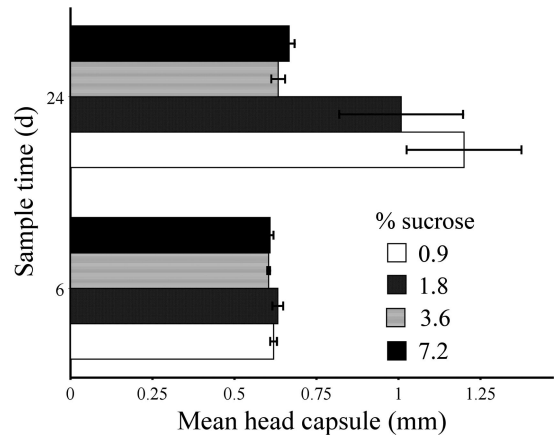

Fig. 3. Mean head capsule width (±1 SD) in larvae reared on diets containing different sucrose concentration assessed 6 and 24 d after hatching. Bars with same letter are not significantly different.

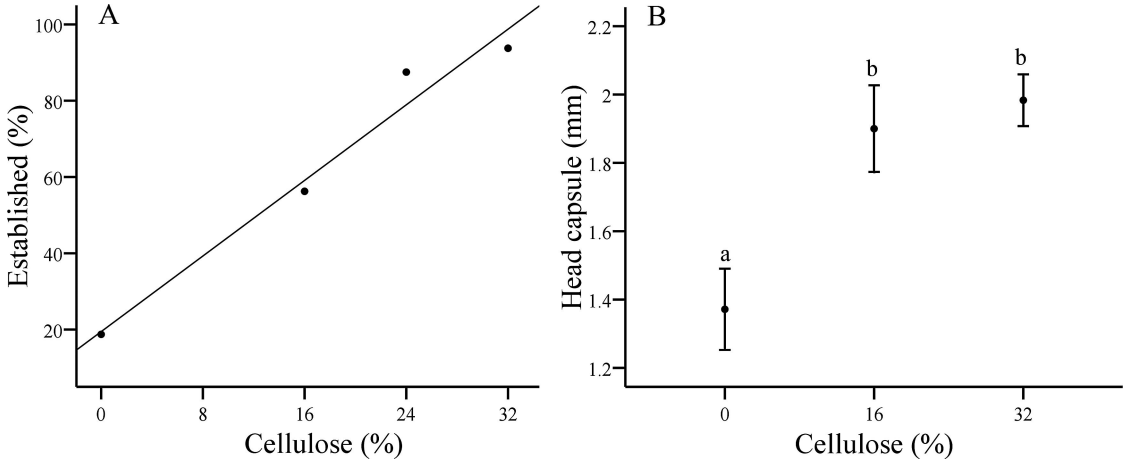

Fig. 4. Effect of cellulose concentration in diet on establishment of larvae assessed 11 d after hatching ($y = 2.478x + 19.464$; $r^2 = 0.969$) (A) and mean head capsule width (±1 SD) assessed 72 d after hatching (B). Means with same letter are not significantly different.

In total, five adults emerged from the cellulose treatment with only 19.23% of the established larvae reaching adulthood. The four eclosed males had variable developmental time with a minimum of 61 d and maximum of 89 d.

Experiment 4. Exclusion of corncob grit from the diet formulation showed no significant differences between the treatment and control in any of the tested variables. Survival rate was 38.76% for the CWD control diet and 36.32% for the corncob grit exclusion treatment, suggesting that corncob grit could be eliminated to reduce diet cost.

Experiment 5. The experimental diet containing soy flour improved first instar survival over the diet containing Pharmamedia ($P = 0.001$). Consequently, soy flour was used in the following bioassay instead of Pharmamedia in all treatments.

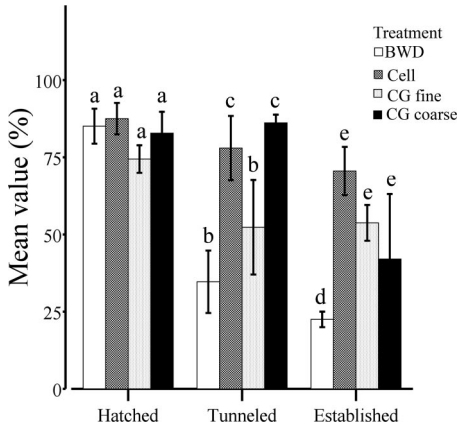

Fig. 5. Effect of bulking agents (cellulose [Cell] and corncob grit [CG] in two different mesh size) in BWD on egg hatching, initial tunneling, and establishment of first instars. Error bars are ±1 SD. Bars with same letter are not significantly different. The percentages of tunneled and established larvae were calculated based on the number of hatched eggs.

Experiment 6. Testing the effects of different carbohydrate sources showed that the initial tunneling of first instars and their establishment did not differ between treatments (Table 3). The mortality of first instars was lower when sucrose was present in the diet (Fig. 6; Table 3). The mortality of immature individuals in advanced stages (larvae >first instar) was not statistically different between treatments (Fig. 6; Table 3). When the head capsule widths of dead larvae in advanced stages were compared, no differences in size were observed (Table 3). Living individuals sampled 130 d after inoculation were either adults that eclosed through the course of the experiment or large larvae. The percentages of weevil survival from all replicates indicate higher survival rate with the sucrose and cellulose diets. The observed pattern across treatments reflects the mortality of the first instars and indicates that the starch diet is inferior.

Analysis on later instars was conducted based on the number of individuals that remained after adjusting for first instar mortality. In this assessment, no significant differences were found (Table 3). Furthermore, ANOVA showed no difference in the number of eclosed adults between treatments (Table 3). Body weight of adult males was significantly affected by the treatments; mean body weight ± SD was $68.244 \pm 1.33e^{-02}$ mg for starch SFWD and $48.466 \pm 1.36e^{-02}$ mg for cellulose SFWD (Table 3). Mean weight of adult females ($67.600 \pm 1.47e^{-02}$ mg) was not affected by the applied treatments (Table 3).

The number of weevils that did not complete development within 130 d was strongly affected by the carbohydrate source in the diet (Table 3). The post hoc test revealed that weevils reared on the cellulose SFWD developed significantly slower than those developing on the starch and cellulose treatments.

The basic recipe for the diet that supports complete development of *H. transversovittatus* was a modified form of the Gast boll weevil diet by using cellulose as filler. The meridic diet presented in Table 2 is still a

Table 3. Results obtained after analysis of data collected in experiment 6 testing the effects of different carbohydrate sources in the diet

Variable	One-way ANOVA			Bonferroni between-groups test		
	F	df	*P*	Sucrose vs. starch	Sucrose vs. cellulose	Starch vs. cellulose
Initial tunneling	1.120	2, 9	0.368			
Establishment	0.538	2, 9	0.538			
Mortality of first instars	33.851	2, 9	<0.001	0.000	0.001	0.008
Mortality of advanced immatures	3.419	2, 9	0.079			
Head capsule size of dead larvae	0.758	2, 86	0.472			
Overall survival	10.311	2, 9	0.005	0.006	1.000	0.020
Survival after first instar mortality	3.769	2, 9	0.065			
Eclosed adults	1.069	2, 9	0.383			
Body wt of males	3.672	2, 31	0.039	0.137	0.522	0.039
Body wt of females	2.989	2, 27	0.065			
Living larvae	15.078	2, 9	0.001	0.909	0.008	0.002

Bonferroni comparison between-groups test was performed to show the relationship between individual treatments (columns under Bonferroni test: sucrose vs. starch, sucrose vs. cellulose, and starch vs. cellulose).

preliminary experimental formulation, but seven sequential generations of insects have been reared using this diet with an average yield of 35% from hatched egg to adult.

Discussion

Study results indicate that the role of sucrose and indigestible carbohydrates varied as the development of *H. transversovittatus* progressed, suggesting a change in functional and nutritional requirements of maturing larvae. Altering sucrose concentrations in the diet did not modify initial tunneling behavior of larvae, indicating the low importance of sucrose in the neonate's burrowing phase. Jolivet (1998) stated that for root feeders, only positive geotropism is needed to dig into the soil. Possibly, initial tunneling of newly hatched *H. transversovittatus* larvae was caused by an innate behavioral pattern such as geotropism rather than substrate attractiveness. However, using different fillers modified initial tunneling in experiment 3.

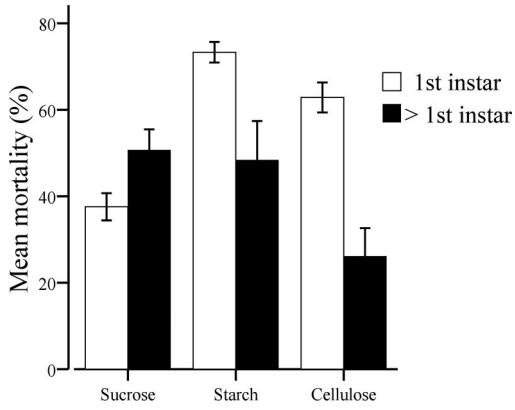

Fig. 6. Mean percentage of mortality of first instars and larvae in advanced stages (>first instar) reared on diets containing different carbohydrate sources. Sample size: n = 171 for sucrose, n = 200 for starch, and n = 181 for cellulose treatments, respectively. Error bars are ±1 SD. Bars with same letter are not significantly different.

Corncob grit is composed primarily of cellulose, chemicellulose, and lignin (Beery and Ladisch 2001), and it has strong absorptive properties. The different mesh (fine and coarse) corncob grit modified diet texture and wetness, which could have attributed to the differences in tunneling success. Egg hatching was not affected by the addition of bulking agents. The different microenvironments created by addition of fillers were well tolerated by eggs, probably as the result of an adaptation to the diverse conditions to which *H. transversovittatus* eggs are exposed in the field. Under natural conditions females oviposit in purple loosestrife shoots, but when stems harden in the late summer, eggs are laid in the soil in proximity to the host plant (Blossey 1993). The bulking agent with largest granule size produced the best tunneling substrate (Fig. 5). This may be the result of the uneven diet surface created by this filler, which enabled newly hatched larvae to gain traction and more easily burrow into the diet. However, this same diet (coarse corncob grit) was the least acceptable for larval establishment. Differences between the initial tunneling rate and the rate of establishment indicated the importance of diet palatability for continuation of feeding and establishment of *H. transversovittatus* larvae. Although only five adults eclosed, this test was highly significant because it represented the first meridic diet capable of producing adult *H. transversovittatus.* Cellulose was treated as a filler because most phytophagous insects cannot use cellulose as a nutrient source due to a lack of enzymes necessary for digestion. The exceptions are cases when symbiotic bacteria or fungi assist with digestion and enable the utilization of otherwise undigestible cellulose (Nation 2002). We did not specifically test the ability of *H. transversovittatus* larvae to digest cellulose, but an addition of antibacterial and antifungal agents (up to three times the final dose) did not affect the survival of larvae through several generations; thus, the use of nutrients was not dependent on the presence of symbionts.

The addition of fillers as a strategy for diet optimization was based primarily on the majority of immature insects having a tendency to consume more when

reared on a well balanced but nutritionally poor substrate until adequate nutrients have been consumed (Singh 1977). It also has been shown that an excess of nutrients can act as antimetabolites (Reinecke 1985) or phagoinhibitors (Sivapalan and Gnanapragsam 1979), resulting in diet repellence. Results of our studies (experiment 1) were consistent with these findings.

Testing the removal of sucrose from the diet or replacing the sucrose with starch in experiment 6 revealed the importance of sucrose for the survival of first instars (Fig. 6). Poor survival of first instars in the two treatments without sucrose may be the result of inhibited feeding activity due to the lack of a feeding stimulus that was provided in the oligosaccharide treatments. It has been previously described that the feeding behavior and nutritional requirements of insects change throughout their development and that these changes may be reflected in food consumption (Barton Browne 1995, Barton Browne and Raubenheimer 2003). A developing *H. transversovittatus* larva in the natural environment changes substrate with maturation. On the host plant, first instar *H. transversovittatus* larvae tunnel into the stem, mining pith before moving below ground. In the cases where eggs are deposited in the soil, hatchlings feed on rootlets and peripherally on the cortex of larger roots until they reach young soft tissues (Blossey 1993). The transition from early to later instars may influence the relative proportion and type of carbohydrates needed in the diet. The highest survival of larvae in advanced stages (other than first instars) was found in the cellulose treatment, suggesting that digestible carbohydrates (sucrose and starch) at the applied concentration produced an unfavorable effect. A similar observation was made by Blossey et al. (2000) where the addition of sugar resulted in significantly lower production of adults.

An additional phenomenon associated with the lack of digestible carbohydrates in the diet was observed in experiment 6. The carbohydrate-poor diet prolonged development, which is consistent with the explanation provided by Danks (1987, 1992) who associated long life cycles to foods with low nutritional value. Development of *H. transversovittatus* from egg to adult on host plants varies from 1 to 2 yr, depending on the time of oviposition (Blossey 1993, Blossey et al. 2000). Diversified life cycles in natural environments with unpredictable or unknown cues are present in many insect species as adaptations to environmental stochasticity (Menu and Desouhant 2002), and these results suggest that diet might be one of the factors responsible for this phenomenon.

Our study showed that host plant material was not a necessary ingredient in an artificial diet for *H. transversovittatus* larvae. Consequently, the production of this agent for biological control of purple loosestrife was considerably cheaper because raw host plant material, the most costly ingredient from the previously published diet (Blossey et al. 2000), was not needed. In the rearing system presented here, only adults for egg production were fed host plants. However, adults can feed and survive on CWD diet (Table 2) for prolonged times. By using the meridic diet presented here, the cost of insect production has been reduced from $3.00 (Blossey et al. 2000) or $2.00–2.50 (Matos 2002) to $ 0.75 per insect. The CWD (Table 2) is still in a preliminary experimental formulation; however, seven sequential generations of insects have been reared using different modifications of this diet with an average yield of 35% from hatched egg to adult. This meridic diet is not as efficient as diet containing host plant material (Blossey et al. 2000), which yields ≈50–60% survived, but recent improvements in other aspects of this diet have increased yield to 70–80% (manuscript in preparation). The research discussed here, however, was instrumental in providing the bases for many subsequent adaptations that have led to significant improvement not only for *H. transversovittatus* but also for successful rearing other root-feeding Coleoptera.

Another major advantage of developing a meridic diet over a diet containing host plant material is the potential acceptance of a generalized diet by multiple species of economic importance. The root weevil diet presented here has supported the development of *Cyphocleonus achetes* (Fahraeus) and *Ceratapion basicorne* (Illiger), and it may serve as a starting diet for the development of rearing systems for other root-feeding insects.

The simplicity of preparation and relatively low cost make this diet excellent for laboratory rearing, and, with some refinement, for insect production on a larger scale.

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